Autoimmunity Reviews 14 (2015) 692–702

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

# Multi-antibody composition in lupus nephritis: Isotype and antigen specificity make the difference

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ARTICLE INFO

*Article history:*
Received 17 March 2015
Accepted 2 April 2015
Available online 14 April 2015

*Keywords:*
Autoimmunity
Systemic lupus erythematosus
Lupus nephritis
Anti-DNA antibodies
Anti-αenolase antibodies
Anti-annexin AI antibodies

ABSTRACT

Research on autoimmune processes involved in glomerulonephritis has been for years based on experimental models. Recent progress in proteomics has radically modified perspectives: laser microdissection and proteomics were crucial for an in vivo analysis of autoantibodies eluted from human biopsies. Lupus nephritis has been the subject of recent independent researches.

Main topics have been the definition of renal autoimmune components in human lupus biopsies; methods were laser capture of glomeruli and/or of single cells (CD38+ or Ki-67+) from tubulointerstitial areas as starting step followed by elution and characterization of renal antibodies by proteomics.

The innovative approach highlighted different panels of autoantibodies deposited in glomeruli and in tubulo-interstitial areas that actually represented the unique autoimmune components in these patients. IgG2 was the major isotype; new podocyte proteins (αenolase, annexin AI) and already known implanted molecules (DNA, histone 3, C1q) were their target antigens in glomeruli. Vimentin was the antigen in tubulo-interstitial areas.

Matching renal autoantibodies with serum allowed the definition of a typical autoantibody serum map that included the same anti-αenolase, anti-annexin AI, anti-DNA, and anti-histone 3 IgG2 already detected in renal tissue. Serum levels of specific autoantibodies were tenfold increased in patients with lupus nephritis allowing a clear differentiation from both rheumatoid arthritis and other glomerulonephritis. In all cases, targeted antigens were characterized as components of lupus NETosis.

Matching renal/serum autoantibody composition in vivo furnishes new insights on human lupus nephritis and allows to refine composition of circulating antibodies in patients with lupus. A thoughtful passage from bench to bedside of new knowledge would expand our clinical and therapeutic opportunities.



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http://dx.doi.org/10.1016/j.autrev.2015.04.004
1568-9972/

**Contents**



## 1. Introduction

Autoimmune renal diseases represent a relevant source of morbidity and mortality in human beings [1]. They include several conditions with different clinical expressions, at the basis of which there is a common mechanism linked to the formation of auto-antibodies versus renal proteins. Renal basement membrane and podocytes are the principal targets but also tubular cells may become the site for autoantibody deposition. Secondary events are complement deposition, mainly C5b-9 [2–6], and activation of podocyte intracellular mediators such as phosphorylated protein kinase C and free oxygen radicals [7]. The imbalance between inflammatory mediators and their inhibitors determines podocyte injury [8,9]. Lupus nephritis and membranous nephritis are the two autoimmune conditions of major interest due to similarities in pathologic pictures (both membranous and class V lupus nephritis present sub-epithelial immune deposits) and potentially they share mechanisms. Anti-GBM is another rare autoimmune glomerulonephritis that is completely entailed by deposition of antibodies versus the NC1 domain of collagen IV causing recruitment of neutrophils and monocytes, destruction of the glomerular capillary wall and crescent formation [10]. While the target antigen and mechanisms of anti-GBM have been elucidated in the past, most of the aspects linking autoimmunity and renal damage in membranous and in lupus nephritis are of only recent evolution and are here reviewed in the light of most recent discoveries. New advances in the field of tissue micro-dissection and protein characterization by mass spectrometry represented the technology keys that allowed a direct in vivo approach to human biopsies. The starting consideration was that only autoantibodies present in the kidney could be considered pathogenic and matching the auto-antigen/antibody renal map with serum antibodies was crucial for developing new concepts on mechanisms and make new biomarkers available for patients with lupus nephritis.

## 2. 'In vivo' renal antibody characterization

### 2.1. General consideration and methodology

A simplified interpretation of the events leading to autoantibody formation and deposition in the kidney considers that renal proteins may become immunogenic. In the case of components normally present in cytoplasm of cells, their dislocation to membrane may also contribute. Cellular mechanisms, potentially involved in human pathologies, are barely evaluated with traditional techniques of analysis and many renal diseases, different in pathogenesis, prognosis and treatment have similar, often undistinguishable, microscopical patterns. Experimental models are helpful but there are cases, such as membranous nephropathy, in which mechanisms in animals do not reproduce human pathology [11–13]. Studies on Heymann nephritis (the experimental model of human membranous nephropathy) were critical to show [11,12,14,15] $IgG_4$ prevalence and complement participation to immune deposit formation, as well as podocyte activation and production of pathogenetic mediators such as reactive oxygen species [2,4,16,17]. However, they did not result in the identification of the human target antigen of membranous nephropathy since megalin, the podocyte antigen in mice, is not expressed in human podocytes. After some decades of substantial no progress, developments of methods for a direct analysis of human tissue [18–22] allowed a direct definition of autoantibodies in human biopsies. Glomeruli (or tubule, or any other structure) can be precisely isolated by laser capture microdissection, a technique that allows the collection of sufficient amount of tissue from the same patient thus permitting a personalized quantitative characterization of different auto-antibodies/antigens. Mass spectrometry evolution is basic to this approach. In this way, the composition of glomerular proteins can be determined, creating a sort of "glomerular proteome map" for different diseases. Antibodies can be micro-eluted as well, allowing the definition of their target antigens, class, isotype and epitope spreading [22].

Evaluation of a potential renal auto-antigen/antibody requires the fulfillment of the following criteria: *1*) the antigen must be expressed in the kidney (glomeruli, tubule) in concomitance with the pathological condition; *2*) the antibody must be eluted from microdissected tissues/cells in vivo; *3*) co-localization of antigen and antibody in immune deposits must be documented by immunohistochemistry and confocal microscopy and, possibly, by immune-electron microscopy; and *4*) elevated circulating antibodies of the same class and isotype should be documented in serum.